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(54) **BETA-ELEMENE, METHOD TO PREPARE THE SAME AND USES THEREOF**

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(57) **ABSTRACT**

This invention provides an anti-cancer composition of high purity beta-elemene extracted from plant sources. This invention also provides for the use of the composition as well as a low cost method to prepare it by multiple passes through the precision distillation tower.

12 Claims, No Drawings

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BETA-ELEMENE, METHOD TO PREPARE THE SAME AND USES THEREOF

This is a 371 of PCT/US98/07341, Filed Apr. 11, 1998, which claims priority of Chinese patent application No. 97103910.0, Filed Apr. 14, 1997, the content of which are incorporated here into this application.

Throughout this application, various publications are referenced and full citations for these publications may be found in the text where they are referenced. The disclosures of these publications are hereby incorporated by reference into this application in order to more fully describe the state of the art as known too the skilled therein as of the date of the invention described and claimed herein.

BACKGROUND OF THE INVENTION

Beta-elemene is a chemical compound that can be extracted from numerous plants. Curcuma Wenyujin Chen et C. Ling, *Curcuma aromatia,* and *Curcuma longa linn* (all belonging to Ziniberaceae) are resources for elemene extraction in China. They grow in tropical areas around the world. In China they are found primarily in Guangdong, Sichuan Fujian, Guangxi and Zhejiang provinces. More than 50 different plants have been found to contain beta-elemene, such as Radix Inulae, Radix Ginseng, E. Wenyujin chen et C. Ling and others.

Beta-elemene can be extracted from the essential oil contained in plants. In China, about 40 different essences were found to contain more than 1% beta-elemene. Higher concentrations are found in the essential oil produced from *Magnolia sieboldii, Citrus junos* leaves and *Aglaia odorata* flower and particularly, *G. Cymbopogon winterianus* Jowitt.

G. Cymbopogon winterianus Jowitt is one of the major plant resources for essential oil production in China. The essential oil contains 1.63-5.21% of beta-elemene and the byproduct of essence extraction i.e. crude product, contains as much as 80% beta-elemene.

Currently China produces more than 2000 tons of oil extracted from the *G. Cymbopogon winterianus* Jowitt annually. This production is over 50% of the world total, and it is one of the major essential oil exports for China. *G. Cymbopogon winterianus* Jowitt provides a rich resource from which large quantities of beta-elemene can be extracted economically.

Beta-elemene has the chemical name:

1-methyl-1-vinyl-2,4-diisoprotenyl-cyclohexane.

Beta-elemene is found in elemene in the various extracts, along with gamma and delta elemene, which are chemical isomers of the beta-elemene. The extracts contain other compounds as well and it is very difficult to isolate beta-elemene from these other compounds using routine isolation techniques.

Preparations made from Curcuma *Aromatica Salisb* (which contains elemene) have been a part of Chinese herbal remedies for centuries. It has been used internally and topically for a wide variety of ailments.

Elemene at various concentrations with other ingredients appears in applications as diverse as a mosquito repellent (see U.S. Pat. No. 5,66,781), burn treatment (see U.S. Pat. Nos. 5,558,914 and 5,384,125) and treatment for Herpes Simplex (see U.S. Pat. No. 5,385,733).

Of particular interest is the anti-tumor characteristics exhibited by elemene.

Yang, H., Wang, X. and Yu, L, Journal article—NHI database ID# 98048492—"THE ANTITUMOR ACTIVITY OF ELEMENE IS ASSOCIATED WITH APOPTOSIS" Chung Hua Chung Liu Tsa Chih;18(3) :169–72 1996, Cancer Institute, Zhejiang Medical university, Hangzhou, China determined that the anti-tumor activity of elemene is associated with cell cycle arrest from S to G2M phase transition and with the induction of apoptosis. They further demonstrated this effect in vitro and in vivo to human and murine tumor cells.

All purity and concentration values in this application are reported by volume. The concentration % in a sample is the volume of beta-elemene divided by the total volume of the sample, multiplied by 100.

Guo,Y T. Journal article—NIH data base ID# 83285582—"ISOLATION AND IDENTIFICATION OF ELEMENE FROM THE ESSENTIAL OIL OF CURCUMA WENYUJIN", Chung Yao Tung Pao; 8(3):31 1983)(the present inventors were co-authors of this paper) described the isolation of elemene from Curcuma Wenyujin and described elemene's anti-neoplastic activities. Beta-elemene in a concentration of 92% was used. The beta-elemene was extracted using chromatography.

Wang J., Zhang H,, and Sun, Y. Clinical trial-Journal article—NIH data base ID#98048551—PHASE III CLINICAL TRIAL OF ELEMENUM EMULSION IN THE MANAGEMENT OF MALIGNANT PLEURAL AND PERITONEAL EFFUSIONS" reported the use of elemene emulsion in the management of malignant effusions.

Shi, J. Journal article—NHI data base ID # 82631581—"Experimental pharmacological studies on the volatile oil of Wen-E-Zhu (Curcuma Aromatica Salisb): Study on the anti-tumor activity of beta-elemene" —Zhongyao Tongbao, Luda Inst. Medical and Pharmaceutical Sciences, Luda, P.R.China demonstrated that the beta-elemene component of elemene exhibited marked anti-tumor activity against murine Ehrlich ascites carcinoma and rat ascitic reticulum cell sarcoma. Diarrhea and weight loss were reported as side effects during treatment. The material used contained beta-elemene 65%, gamma and delta-elemene 20%, and impurities 15%. The beta-elemene was extracted by chromatography.

Fu, N. W. Journal article—NIH data base ID #84282970—"ANTITUMOR EFFECT AND PHARMACOLOGICAL ACTIONS OF BETA-ELEMENE ISOLATED FROM THE RHIZOME OF CURCUMA AROMATICA", Chung Yao Pao; 9(2) :35–9 1984 reported on the anti-tumor effect of beta-elemene, one of the monomers comprising the elemene isomer. As in Shi. J article above, the material used contained beta-elemene 65%, gamma and delta-elemene 20%, and impurities 15%.

In December 1993 elemene was designated as a Chinese national Class II new drug. In February 1994 the anti-cancer effect of elemene was confirmed by the health authority of the P.R. of China.

During the 2 year trial of the drug in China, it was determined that elemene has not only the ability to manage malignant chest and abdominal ascites, but also had beneficial effects on brain tumors (neuroglioma), liver and esophageal cancer.

The elemene has apparent anti-cancer activity on mouse Ehrlich Ascites Carcinoma (EAC) and mouse leukemia P388, L_{1210}, ARS and rat YAS etc. From the Chinese phase I, II and III clinical trials, it was proved that the elemene exhibits some control over cancerous chest and abdominal ascites and surface tumors. The total effect rate was about 69%. It should be noted that 27% of the cerebral carcinoma patients reached CR level with a combination of the elemene and local chemotherapy in the expanded clinical trials.

SUMMARY OF THE INVENTION

The research conducted demonstrated that the beta-elemene component of elemene had significant anti-tumor characteristics and that the primary mechanism was the induction of apoptosis in the tumor cells.

Previous researchers produced beta-elemene by chromatography, which produced only small quantities of beta-elemene at high cost. The maximum purity attained had been 92%.

In addition, work performed in the Department of Embryology of the Dalian Medical University revealed that beta-elemene is able to pass through the brain-blood barrier (BBB) and thus reach tumors within the brain.

It is an object of the present invention to provide for an anti-cancer drug wherein the active ingredient is beta-elemene.

This invention relates generally to compositions of beta-elemene and method of producing higher concentration than previously possible, produced at reasonable cost using abundant plant sources; pharmaceuticals containing beta-elemene and application of the pharmaceuticals to treat various malignant diseases.

Since indications for the pharmaceutical composition of beta-elemene at the present time are mainly for malignant disorders it is an object of the invention to provide a method for applying the pharmaceutical to treat various malignancies, especially neuroglioma and solid tumors.

Having identified the beta-elemene monomer as a component of elemene with a strong anti-tumor activity, it is a purpose of the present invention to provide for a method to produce it in quantity and with sufficient purity to reduce the side effects from any impurities, and provide a standard dosage. As a result, the governmental requirements for pharmaceuticals with respect to analytical definition and reproducible composition, independent from the variable composition of the starting material from any of several plants can be fulfilled.

A further object of the invention is to provide a beta-elemene composition of high concentration (96.4–97.2%). A composition with highly concentrated effective content and minimum impurities is required in many countries with high pharmaceutical standards which are not usually met by simple extracts since the norms apply to pure substances. Until now it has not been possible to prepare such high concentrations of beta-elemene from the plant sources.

Another advantage of the highly concentrated bete-elemene is the reduced amount that must be dosed to be effective.

An additional advantage of highly concentrated beta-elemene is the further removal of inactive substances. The extensive removal of inactive accompanying substances enhances the safety of the pharmaceutical, since the simpler composition of the active component concentrate facilitates a more precise analytical determination of the main components and detection of potential impurities.

It is a further object of the invention to provide a delivery means for effective levels of the beta-elemene to reach surface and internal tumors.

It is a further object of the invention to provide a low-cost high-volume means of production of beta-elemene.

The present invention is the use and preparation of beta-elemene that is characterized by a purity of more than 96%. The invention involves the preparation methods and the composition of an anti-cancer drug, especially with the

preparation methods and the composition of an anti-cancer drug in which the beta-elemene is the effective ingredient.

DETAILED DESCRIPTION OF THE INVENTION

China produces 2000 tons of oil extracted from *G. Cymbopogon winterianus* Jowitt. The oil contains 1.63–5.21% beta-elemene. In the process of extracting the oil for its essence a by-product, called "crude product", is produced. This crude product has a higher concentration of beta-elemene than the original plant material, as high as about 80%. This crude product provides an inexpensive, abundant source of feed material for this new process.

This invention provides a composition of 96.4–97.2% beta-elemene.

The other ingredients are

gamma-elemene	0.5%
elemenol	0.8–1.2%
Copane	1.3–1.7%
Isofuranogermacrene approx.	0.2%

This invention provides a composition of at least 96.4% beta-elemene.

This invention provides the above compositions having components extracted from a group of over 40 plants known to have useful levels of beta-elemene.

This invention provides an injectable formulation containing the above compositions. The injectable formulation may then be administered to the subject via different routes, such as intravenous injection, intramuscular injection, intradermal injection, peritoneal injection and injection into solid tumor.

This invention provides the above compositions that can be added into cream, ointments or presence in raw materials to prepare the same.

This invention provides a method for obtaining a beta-elemene composition using a distillation tower to separate the crude material into fractions, the tower being specifically constructed (the precision distillation tower) to produce beta-elemene at a concentration of 96.4–97.2% and at very low cost.

This is the first time that the precision distillation tower is used to produce beta-elemene. It is achieved through the multi-fractionation of the crude material in the precision distillation tower.

The precision distillation tower to be used is designed and manufactured specifically for this purpose. A tower has a height of 1.4 meters, a tower diameter of 6 cm and the tower interior is fitted with as many 3×3 mm hollow 120 mesh stainless steel cylinders as can be fit within it. The number of plates within the tower is 40.

This invention utilizes at least one of the following as the source of the plant material: *G. Cymbopogon winterianus* Jowitt, *zhangzhou Aglaia odorata* flower, *Fuzhou Aglaia odorata* flower, *Chunging Aglaia odorata* flower, *Chunging Aglaia odorata* leaves, *Zhangzhou Aglaia odorata* leaves, *Yibin geranium* leaves, *Kunmin geranium* leaves, *Litchi chenensis cinnamomifolium,* dry *Lauris nobilis, Citrus limona* leaves, *Vitis vinitera* grape leaves, *Clausena lansium* leaves, *Fortunella margarita* leaves, *Fortunella oborata,* C. Wenyujin Chen, and *Magnolia sieboldi.*

This invention provides for a method comprising the following steps:

(A) obtaining as a plant source at least one of the following plants: *G. Cymbopogon winterianus* Jowitt, *Zhangzhou Aglaia odorata* flower, *Fuzhou Aglaia odorata* flower, *Chunging Aglaia odorata* flower, *Chunging Aglaia odorata* leaves, *Zhangzhou Aglaia odorata* leaves, *Yibin geranium* leaves, *Kunmin geranium* leaves, *Litchi chenensis cinnamomifolium,* dry *Lauris nobilis, Citrus limona* leaves, *Vitis vinifera* grape leaves, *Clausena lansium* leaves, *Fortunella margarita* leaves, *Fortunella oborata,* C. Wenyujin Chen, and *Magnolia sieboldi.*

(B) extracting oil from said plant source to produce crude product;

(C) loading said crude product into the distillation tower to separate said crude product into fractions; and

(D) collecting the fraction with the highest concentration of beta-elemene, thereby producing a composition with a concentration of about 95t beta elemene. The harvest rate for this fraction is about 63% of the input.

The beta-elemene composition produced in step (D) is further concentrated by a second fractionation comprising the following additional step:

(E) loading the composition comprising about 95% beta-elemene into the tower to separate into fractions; and collecting the fraction with the highest concentration of beta-elemene, thereby producing a composition of beta-elemene with a concentration of 96.4–97.2%. The harvest rate is about 60%.

In the preferred embodiment, this invention provides for the method of obtaining beta-elemene from its plant sources comprising the steps of:

The First Fractionation

(a) Obtaining the crude product that is produced as a by-product of elemene essence extraction (such as by the Essence Chemical Factory-China);

(b) Loading 2000 g of crude product containing more than 80% beta-elemene into the precision distillation tower. The precision distillation tower has a height of 1.4 meters and a diameter of 6 cm. The tower cavity is fitted with as many hollow 3 mm×3 mm 120 mesh stainless steel cylinders as can be fit inside. The number of plates within the tower is 40;

(c) Establishing a vacuum of not less than 2 mmHG, usually between 2–5 mmHg with a temperature range from 86 to 93 degrees C. and a reaction time of about 10 hours. There are 3 different temperature ranges for fractionation; 86–88 degrees C., 89–90 degrees C. and 91–93 degrees C. The fraction of 89–90 degree C. has the highest concentration of beta-elemene and is collected. The harvest rate for the fraction of 89–90 degrees C. is about 63% of the input. The purity at this point is about 95%;

For the second fractionation

(d) Take 2000 g of the fractionation previously collected from the first fractionation with a purity of 95% and repeat the process using the same parameters as the first fractionation. During the fractionation use 3 temperature ranges: 86–88 degrees C., 89–90 degrees C. and 91–93 degrees C. The fraction from the 89–90 degree C. range has the highest concentration of beta-elemene and is retained. The harvest rate for the second fractionation is 60% and the purity of the beta-elemene is 96.4–97.2%;

(e) The residue remaining in the tower and the fractions at 86–88 and 91–93 degrees C. can be further refined or recycled. Recycle the unused fractions from the first pass together with the remaining oil extract in the tower which went through two fractionations by loading the tower with these materials. Steps (a) through (d) are repeated and the fraction at 89–90 degrees C. is collected. The harvest rate is 20%.

If desired the fractionation can be repeated further, using these same steps.

The final product from the precision distillation contains:

beta-elemene	96.4–97.2%
copane	1.3–1.7%
Elemenol	0.8–1.2%
gamma-elemene	0.5%
Isofuranogermacrene	0.2%

Beta- and gamma elemene have similar structure and anti-cancer activities.

Since the fractionation is conducted in a sealed container there is no environmental pollution.

The beta-elemene can be used in any pharmaceutically 10 suitable carrier.

In the preferred embodiment, prepare the pharmacological preparation from the beta-elemene composition by the following steps:

(1) Mix the beta-elemene as obtained above with phosphatide and cholesterol, heat to 80 degrees C. until the mix melts down and becomes clear.

(2) Dissolve NaH2PO4 and Na2HPO4 in water and heat to 80 degrees C.

(3) Place the mixtures from above steps (1) and (2) into a high speed emulsifier until the mixtures become emulsive injection.

(4) Filtrate the emulsive injection through a G4 sintered glass funnel, and check the particle size.

(5) Fill the checked emulsive injection into ampules and seal them. Sterilize at 100 degrees C. for 40 minutes to yield the final product.

For the purposes of this invention "pharmaceutically suitable carriers" means any of the standard pharmaceutical carriers. Examples of suitable carriers are well known in the art and may include, but not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solutions, phosphate buffered saline containing Polysorb 80, water, emulsions such as oil/water emulsion, and various type of wetting agents. Other carriers may also include sterile solutions, tablets, coated tablets, and capsules.

Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well known conventional methods.

Research Results

Using the described procedure to prepare the beta-elemene anti-cancer drug, the following results were obtained from animal pharmacodynamic studies:

(A) Ascites carcinoma: intra-peritoneal injection of the beta-elemene has a stable therapeutic effect on EAC and S_{180} ascites cancer. The life prolongation rates are 85–310% and 90–321% respectively. The life prolongation rate for hepatic ascites carcinoma is 103–224%.

(B) Solid tumor; The beta-elemene injection has some inhibition effect on S_{180} solid tumor. The inhibition rate is 33–59%. The inhibition rate for hepatic solid tumor is 10–30%.

(C) Murine leukemia L_{1210}: The beta-elemene injection has obvious therapeutic effect on L_{1210} leukemia. The life prolongation rate was 47–289%.

(D) Neuroglioma: It was indicated in the pharmacodynamic study of cerebral neuroglioma using murine sub-renal capsule model that the inhibition rate was 50–70%.

(E) There was no measurable bone marrow inhibition or reduction of white blood cell count, etc which are common side effects of conventional anti-cancer drugs.

Based on the findings of these experiments, the inventors suggest that beta-elemene has beneficial effects on the treatment of ascites carcinoma, solid tumor, neuroglioma, murine leukemia etc. with no obvious side effects observed. They further conclude that beta-elemene was suitable for development as a national (China) class I new drug for human cancerous ascites, neuroglioma and solid tumor, and for research in its use to be continued.

Human Data

In two instances, patients at the Second Affiliated Hospital of the Dalian Medical University were treated with the beta-elemene composition as an emulsive injection. The medical records were kept at that hospital.

Patient I: (Hospitalization #3106) A 25 year old female Patient had headache, right side arm and leg weakness and hemiplegia. She was diagnosed by CT scan as having a thalamic glioblastoma. The tumor size was 3.8×5.3×4 cm. The patient was hospitalized for surgery in July 1995. 80% of the tumor was excised, but it grew back to the full size within two weeks after the operation. A beta-elemene emulsive injection was employed through 600 ml cervical artery intubation and 400 ml intravenous drip alternately once per day. The growth of the tumor was quickly controlled, but on CT scan there was no apparent reduction in tumor size. The patients symptoms improved and she survived for 11 months with the tumor.

Patient II: (Hospitalization Number 3679) A 65 year-old female

The patient had headache, vomiting and gait problems due to lung cancer. A CT scan indicated that the lung cancer had resulted in multiple metastasis to the brain. The tumor in the right cerebellum was 2.8×4.0×3.1 cm. The size of a tumor in the left ganglion basal zone was 0.5×1.5×1.6 cm. Due to the lung metastasis and the age of the patient, surgery was difficult to perform. The patient was hospitalized in mid November 1996.

It was estimated that she would not survive longer than a month. The patient was treated with beta-elemene as prepared herein. The patient received the same regimen as patient I. Her condition improved, the headache was reduced. The vomiting stopped. In the middle of December 1996 the patient had another CT scan. The size of the tumor was reduced. In March 1997 the patient requested to leave the hospital as the lung cancer spread to the rib and lymph nodes, along with the pathological left forearm fracture. The prolongation of the life of the patient was more than 4 months, based upon previous experience with patients at that stage of illness.

We claim:

1. A method for producing a composition containing beta-elemene from a plant source using a distillation tower comprising the following steps:

(a) obtaining said plant source;

(b) extracting oil from said plant source to produce a crude product;

(c) loading said crude product into a distillation tower to separate said crude product into fractions; and

(d) collecting the fraction with the highest concentration of beta-element, thereby producing a composition of beta-elemene, wherein said tower has a height of 1.4 meters and a diameter of 6 cm; wherein said tower has an inside and outside surface, wherein said inside surface defines a cavity; and wherein said cavity is fitted with as many 3mm×3mm hollow 120 mesh stainless steel cylinders as will fit therein.

2. The method for producing a composition of beta-elemene according to claim **1** further comprising the steps:

(e) loading said tower with said fraction collected in step (d);

(f) drawing multiple fractions; and

(g) collecting the fraction with the highest concentration of beta-elemene, thereby producing a composition of beta-elemene.

3. The method according to claims **1** or **2**, wherein said plant source is at least one selected from the group consisting of *G. Cymbopogon winterianus* Jowitt, *Zhangzhou Aglaia odorata* flower, *Fuzhou Aglaia odorata* flower, *Chunging Aglaia odorata* flower, *Chunging Aglia odorata* leaves, *Zhangzhou Aglaia odorata* leaves, *Yibin geranium* leaves, *Kunmin geranium* leaves, *Litchi chenensis cinnamomifolium,* dry *Lauris nobilis, Citrus limona* leaves, *Vitis vinifera* grape leaves, *Clausena lansium* leaves, *Fortunella margarita* leaves, *Fortunella odorata,* C. Wenyunjin Chen, and *Magnolia sieboldi.*

4. The method for producing a composition of beta-elemene according to claim **3** further comprising the following steps:

(a) establishing a vacuum in said tower of 2–5 mmHg;

(b) heating said tower to a temperature range of 86–93° C.; and

(c) using a reaction time of about 10 hours.

5. The method for producing a composition of beta-elemene according to claim **4** wherein said vacuum is 2 mmHg.

6. The method for producing a composition of beta-elemene according to claim **5** further comprising:

(a) a first fractination comprising the steps of:

producing a fraction at a temperature range of 86–88° C.;

producing a fraction at a temperature range of 89–90° C.;

producing a fraction at a temperature range of 91–93° C.;

collecting said fraction produced at said temperature range of 89–90° C. to obtain a first composition;

removing any residual material remaining in the tower; and

(b) a second fractionation comprising the steps of loading said first composition produced at said temperature range of 89–90° C. into said tower;

producing a fraction at a temperature range of 86–88° C.;

producing a fraction at a temperature range of 89–90° C.;

producing a fraction at a temperature range of 91–93° C.; and

collecting said fraction produced at said temperature range of 89–90° C., to produce a second composition of beta-elemene.

7. The method of producing a composition of beta-elemente according to claim **6** further comprising loading

the fractions collected at 86–88° C. and 91–93° C. into said tower and repeating the steps of claim **6**.

8. The method according to claim **6** further comprising:

combining said fractions produced at said temperature ranges of 86–88 and 91–93° C. with the residue remaining after fractionation;

loading said tower with said combination; and

repeating the steps of claim **6**.

9. The method for producing a composition of beta-elemene according to claim **6** wherein said plant source is *G. Cymbopogon winterianus* Jowitt.

10. The method for producing a composition of beta-elemene according to claim **9** wherein said composition of beta-elemene comprises at least 96% beta-elemene.

11. The method of producing a composition of beta-elemente accrding to claim **10** wherein said composition of beta-elemene comprises between 96.4 and 97.2% beta-elemene.

12. The method of producing a composition of beta-elemente accrding to claim **10**

wherein said composition comprises:

about 96.4–97.2% beta-elemene;

about 0.8–1.2% elemenol;

about 1.3–17% copane;

about 0.5% gamma-elemene; and

about 0.2% isofuranogermacrene.

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